NOTES ON THE NATURE OF "ARTIFICIAL PARTHENOGENESIS" IN THE EGG OF PODARKE OBSCURA.

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The results obtained by Lillie[1] on the phenomena accompanying artificial parthenogenesis in *Chætopterus* led me to repeat the experiments, using *Podarke obscura*, with the idea of determining if similar results appear in this genus. I was much interested, also, in the question whether cleavage produced by chemical stimulation bears any resemblance to the normal cleavage; the latter, in this form, having been carefully followed.

That *Podarke* eggs would develop into ciliated embryos in this way was first determined by Dr. A. W. Greeley, to whose courtesy I am indebted for suggestions in the use of his method. This was treatment for one hour with a mixture of 20 c.c. of a 2½ nKCl solution + 80 c.c. of sea water. They were then transferred to sea water, and, after a variable length of time, began to divide. A solution of 22½ c.c. of 2½ nKCl solution + 77½ c.c. of sea water was also used, but gave less satisfactory results than the other. Through lack of material I did not use any other solutions, and experimented only with unfertilized eggs. This leaves my observations very incomplete, but the results thus far obtained seem worth recording.

Controls were kept in each experiment. A few of the control eggs, after twelve hours or more in sea water passed into what might be termed, by a superficial observer, a "morula" stage. The cytoplasm breaks up into a great many small globules with no trace of nuclear division, the chromatin lying generally in one globule, which is usually larger than the others. These never develop further, and there was never any trace of nuclear cleavage or ciliated embryos in the control eggs. A similar breaking down into a "morula" occurs in unsuccessful experiments among

[1] F. R. Lillie, Differentiation without Cleavage in the Egg of the Annelid *Chætopterus pergamentaceus*. Archiv f. Entwick. d. Organismen, 14 Bd., 3 and 4 Hft.

the eggs treated with the KCl solution and a few usually appear among the ciliated embryos of a successful experiment.

When laid, the egg of *Podarke* passes into the stage of the first polar spindle, and remains in this condition until fertilized. It is not at all uncommon to find among late cleavage stages eggs which have escaped fertilization, and invariably they show the first polar spindle. Treatment with the KCl solution apparently does not stimulate the egg to form the polar globules, for I have not been able to find any of the latter, and in the one-cell stages found in my preparations the chromatin is either packed into a small, deeply staining mass, or scattered through the cytoplasm. Apparently, though I have not as yet sufficient evidence to demonstrate the accuracy of this conclusion, if the causes operating on the egg are such as to produce the former condition, the further change, if any occurs, is purely cytoplasmic in its character, while if the second condition appears, the egg may

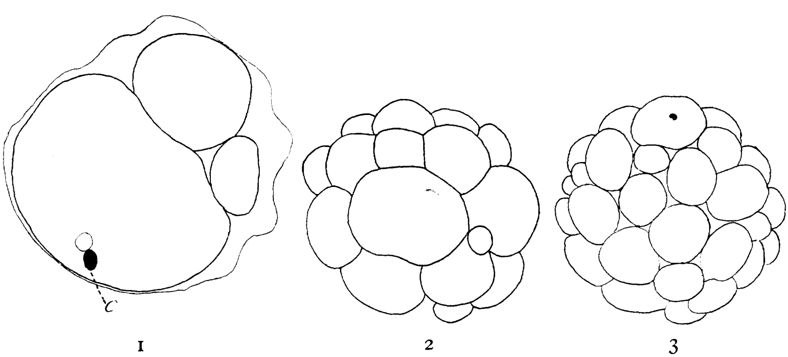


FIG. 1. Division of cytoplasm without nuclear division. *C.* Chromatin. Preserved material.

FIGS. 2 and 3. Like Fig. 1 but showing a more complete division of the cytoplasm. Preserved material.

subsequently divide by a nuclear as well as a cytoplasmic division.

As illustrations of the former case, see Figs. 1 to 3.[1] These were drawn from material preserved two hours and twenty minutes after the transfer to sea water. At this time the eggs were in active

[1] A membrane is present in all of these eggs, but is represented only in Fig. 1. The drawing from preserved material shows a wider space between the egg and the membrane than exists during life.

amœboid movement, though, as the egg membrane is closely applied to the surface of the egg, these movements are not as noticeable as in other animals. They were, however, sufficient in amount to make camera drawings impracticable. The preserved material shows that the cytoplasm is apparently broken up into distinct globules, though from the appearance presented by the living egg I think it altogether probable that they are really in communication with one another, these "globules" being really amœboid processes. As seen in the drawings, these preparations bear a superficial resemblance to a cleaving egg. There is, however, absolutely no resemblance to the normal cleavage, and a study of the stained material shows that the division is one involving only the cytoplasm, the chromatin being massed together in one of the spheres. This sphere is usually, though not always, larger than the others. (In the egg from which Fig. 3 was drawn, the chromatin, except the small bit seen in one of the upper cells, was in a large cell on the side of the egg opposite that from which the drawing was made.)

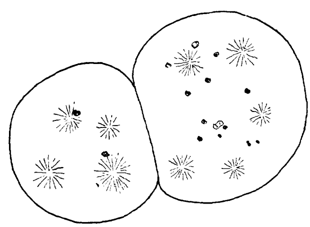


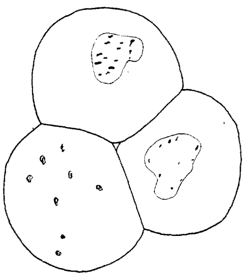
FIG. 4. "Two-cell" stage. The nuclear matter is scattered through the cytoplasm. Preserved material.

In the second of the above-described cases, cleavage involves both nucleus and cytoplasm. In Fig. 4 several asters are plainly to be seen in each cell, which leads to the suggestion that possibly it is upon the presence or absence of these that the differences in nuclear activity are dependent. Division of these eggs is apparently complete. In the two-cell stage the greatest possible variations in size between the two cells appear, the division being sometimes nearly equal, as in Fig. 4 and again very unequal. I have no reason to believe that this division is karyokinetic. It seems rather as if one of the cells is to be regarded as a lobe of protoplasm which contains some of the nuclear material, and may later become completely divided by a membrane from the other cell. Instead of two cells, a trefoil stage may appear.

What I interpret as later developments of the stage just described are shown in Figs. 6 and 7. These divisions are appar-

ently karyokinetic, but in none of them is there the slightest resemblance to the normal cleavage pattern.

Fig. 8 is of especial interest. In about twelve hours after transfer from the salt solution to sea water, there will be found



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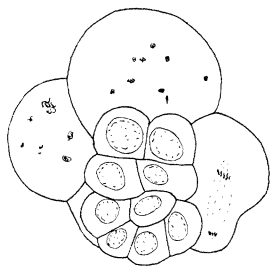

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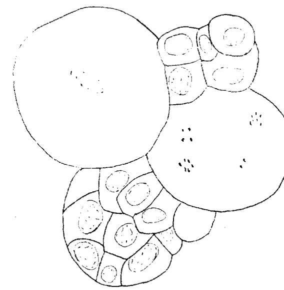

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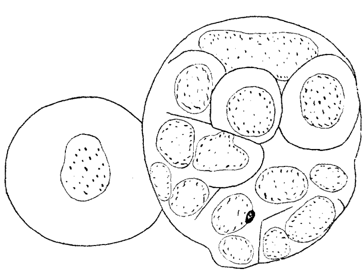
FIG. 5. Trefoil stage. Preserved material.

FIGS. 6 and 7. Later development of stage shown in Fig. 4. Preserved material.

numbers of swimming embryos, looking much like the half embryo formed by destroying one blastomere of the two-cell stage. It is apparently formed from an embryo like that of Fig. 4 by a development of one cell, while the other remains undivided. Since it seems probable that the latter is to be regarded as a cell with an "extra ovate" attached, rather than a true two-celled



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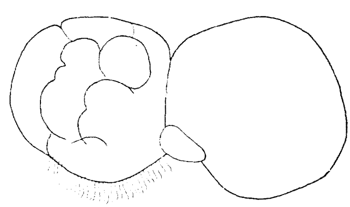

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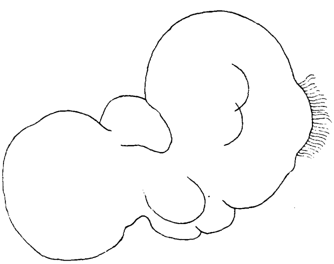
FIG. 8. Embryo produced by development of half of embryo shown in Fig. 4. Preserved material.

FIG. 9. Embryo like that of Fig. 8. Living material.

stage, we might say that here either the extra ovate or its fellow developed, while the other part remained undivided. These divided portions put out cilia, and swim about in the water, carrying the undivided portion with them. Later, they break away

from the undivided portion and swim independently, forming "dwarf embryos." So far as I can discover, their cleavage has no resemblance to the normal. Their abnormal character is also indicated by their nuclei, which have a very characteristic appearance, resembling closely those of abnormal eggs which occasionally appear among a normally fertilized lot. The nuclei are much larger and stain much more intensely than the normal ones. Fig. 9 is from a living egg of this sort, and shows the arrangement of the cilia.

Besides these ciliated embryos are others, showing no indication of cleavage, but with well-developed cilia. Fig. 10 shows



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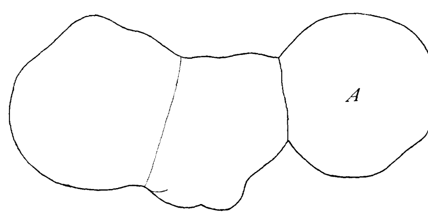

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FIG. 10. Ciliated, unsegmented embryo. Living material.
FIG. 11. Unsegmented embryo formed by fusion. Living material.

one of these where the whole embryo is very much distorted, but with a well-marked tuft of cilia. Staining with aceto-carmine after the above drawing was made, gave no indication of cleavage in this embryo.

Fig. 11 shows a case of fusion of at least two eggs. This was from an embryo slowly rotating around the end *A* as a pivot. I was unable, however, to discover the distribution of the cilia causing this movement.

Fig. 12 shows a still more interesting condition. Here, also, there is apparently a fusion of at least two cells, but there is no trace of cleavage. At *n* is possibly a nucleus. Around one portion of the fused mass is a ring of cilia, occupying very much the position, with respect to the cell, of the prototroch in its relation to the trochophore. Not only are the cilia present, but around the embryo, underneath the ciliated band, is an area free

from the ordinary pigment of the rest of the cell, but containing granules of a faint yellow color, agreeing in this respect exactly with the prototroch band of the normal trochopore. In this embryo there is, then, not merely a differentiation—without cleavage—of cilia, but of the characteristic protoplasm accompanying these cilia—or, in other words, we have here, in the unsegmented embryo, not merely a differentiation of cilia, but a differentiation of *prototroch cilia*. Later, fused masses like those just described are apt to break down, setting free the ciliated fragments, and by the twentieth hour the culture will be swarming with ciliated fragments derived from this source and from the breaking away of the "dwarf" described above. These, of course, have not even a superficial resemblance to normal embryos.

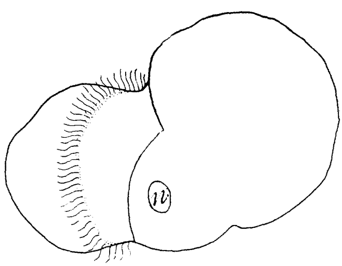

FIG. 12. Unsegmented embryo formed by fusion with well-differentiated band of cilia. Living material.

Fusion phenomena are rare in *Podarke* as compared with *Chætopterus*, the largest fused masses I have found containing not more than six eggs.

SUMMARY.

1. After treatment with 2½ nKCl solution, eggs of *Podarke obscura* exhibit various cleavages and pseudo-cleavages.

2. The pseudo-cleavages involve only the cytoplasm and not the nucleus, and have no resemblance to the normal cleavages.

3. The cleavage involves both nucleus and cytoplasm, and may give rise to a ciliated embryo. The cleavage in this case, however, does not resemble the normal.

4. Ciliated embryos may arise without cleavage. The differentiation may be carried very far in such cases. This is especially noticeable in the character of arrangement of cilia.

5. Fusion of embryos may occur, but in the solution mentioned, much fewer cells unite than in, *e. g.*, *Chætopterus*.

MARINE BIOLOGICAL LABORATORY,
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